

Monovalent Ion Stimulated Adenosine Triphosphatase From Oat Roots¹

James Fisher and T. K. Hodges

Department of Horticulture, University of Illinois, Urbana, Illinois 61801

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Abstract. Monovalent ion stimulated ATPase activity from oat (*Avena sativa*) roots has been found to be associated with various membrane fractions (cell wall, mitochondrial and microsomal) of oat roots. The ATPase requires Mg^{2+} (or Mn^{2+}) but is further stimulated by K^+ and other monovalent ions. The monovalent ions are ineffective in the absence of the divalent activating cation. The ATPase has been described with respect to monovalent ion specificity, temperature, pH, substrate specificity, and Mg^{2+} and K^+ concentrations. It was further shown that oligomycin inhibits a part of the total ATPase activity and on the basis of the oligomycin sensitivity it appears that at least 2 membrane associated ATPases are being measured. The mitochondrial fraction is most sensitive to oligomycin and the microsomal fraction is least sensitive to oligomycin. The oligomycin insensitive ATPase appears to be stimulated more by K^+ than the oligomycin sensitive ATPase.

It was further shown that per gram fresh weight of roots, approximately 0.7 to 0.8 μ moles of K^+ were absorbed per μ mole of K^+ stimulated ATP hydrolysis. This result was obtained for a variety of K^+ concentrations and was taken to mean that sufficient membrane associated ATPase exists to account for K^+ transport in the oat roots.

It is well known that ion transport in plants requires respiratory energy (22, 24, 29). It is also felt by most investigators, on the basis of kinetic studies (8, 13), that ions traverse membranes in association with some type of 'carrier' substance. However, the actual energy source for transport, as well as the mechanism of energy transfer to the transport process itself, are not known. With respect to the energy source, it has been postulated that either electron transfer phenomena or ATP could represent the forms of energy utilized in transport processes (29). Unequivocal evidence in support of either possibility simply does not exist. It has been demonstrated, however, that ATP can provide the energy for Ca^{2+} transport in plant mitochondria (7, 17, 19, 20), but whether a similar reaction exists at the plasmalemma is unknown. The fact that oligomycin inhibits ion absorption by roots (18, 21) at concentrations having no effect on root respiration (18), suggests that a similar ATP-driven reaction may occur at the cell surface.

One experimental approach which has clarified both the nature of the energy source and the nature of the energy transduction process in animal cells and tissues has involved the isolation and characterization of a membrane associated enzyme which uses ATP as its substrate (ATPase) and which is activated by the ions which are actively transported by the cells in question (2, 33). Thus it has been found that in cells which possess a coupled active transport

of Na^+ and K^+ , the cell plasma membrane contains a Mg^{2+} requiring, $Na^+ + K^+$ stimulated ATPase. The fact that the cardiac glycoside, ouabain, specifically inhibits the coupled transport of Na^+ and K^+ and also inhibits the $Na^+ + K^+$ stimulated ATPase clearly illustrates the intimacy of this enzyme with the transport process (33).

Several recent reports have been made of the existence of ion sensitive ATPases from plant tissues (1, 3, 4, 5, 15, 27). However, the effect of inorganic ions on the plant ATPase is reported to either slightly stimulate or inhibit activity and it is not clear whether the enzymes being measured are of sufficient activity to account for the transport of ions. We have observed a monovalent ion stimulation of an ATPase from oat roots and this report describes some of our initial findings. The enzyme exhibits some specificity toward monovalent ions and it would appear that sufficient K^+ stimulated ATPase exists to account for the observed rates of K^+ influx in roots.

Materials and Methods

The roots from 3 to 5 day old, dark grown, oat (*Avena sativa* var. Goodfield) seedlings were used in all experiments. The dry seeds were placed between layers of cheesecloth on a stainless steel screen which was supported by a 4 liter beaker containing 2.5 liters of 1 mM $CaCl_2$. The temperature was $25^\circ \pm 3^\circ$ and vigorous aeration was provided. The distance between the solution level and the screen was about 10 cm. This method of growing the

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seedlings resulted in better germination and generally whiter, healthier looking roots than could be obtained using the more conventional solution culture method.

Prior to the experiment the roots were excised, washed 3 times in distilled water and chilled prior to homogenizing in an ice-jacketed mortar and pestle. The roots were ground in a medium consisting of 0.25 M sucrose, 0.003 M EDTA, pH 7.5 with tris. The debris was strained through 2 layers of cheesecloth and various fractions were sedimented by centrifuging at 1°. The fraction referred to as cell wall was sedimented at 1500g for 10 min, the mitochondrial fraction between 1500g for 10 min and 12,000g for 15 min and the microsomal fraction was obtained by centrifuging the mitochondrial supernatant solution at 100,000g for 90 min. The cell wall and mitochondrial fractions were washed one time with 0.25 M sucrose and the pellets were finally suspended in 0.25 M sucrose. For the total ATPase distribution experiments the washing and suspending solutions contained 0.25 M sucrose, 0.001 M EDTA, pH 7.5 with tris and the crude extract and soluble fractions were diluted with 0.25 M sucrose to give the same concentrations of EDTA and tris as the membrane fractions.

The ATPase assay was carried out in a total volume of 4 ml and the reaction mixture consisted of ATP (the tris salt of ATP was used in all experiments except those indicated in the tables) pH 7.5 with tris and the various ions to be tested. Experiments were initiated by the addition of 0.5 ml of enzyme containing approximately 0.1 mg protein. Incubations were 15 to 30 min at 38° in a shaking water bath. The reactions were terminated by the addition of trichloroacetic acid (final concn of 5%). The protein was removed by centrifugation and the P_i liberated from ATP was determined by the method of Fiske and Subbarow (12). Protein was determined according to Lowry *et al.* (23).

The extraction and analysis of phospholipid was as described previously (16).

Potassium influx was determined using ^{86}Rb as a tracer for K^+ as described previously (6). Briefly, the roots were excised and washed 3 times in distilled water prior to the absorption. The roots (0.5–1 g) were bound in bags of cheesecloth as described by Epstein *et al.* (9) and held in cold

deionized water until initiation of the experiment. The roots were then placed in distilled water at 30° for 45 sec prior to immersing them in 1 liter solutions of various concentrations of KCl (^{86}Rb). The absorption solutions were maintained at 30° and aeration was provided. After 30 min of absorption the roots were rinsed briefly in a cold (2°) exchange solution consisting of 5 mM KCl, 0.5 mM CaSO_4 and then placed into 4 liters of a fresh, cold solution of the same composition for an additional 30 min. The roots were finally rinsed thoroughly with distilled water, blotted, weighed and ashed in planchets at 500°. The ash was moistened with 0.25 ml of Photoflo, dried and counted for radioactivity.

The various subcellular membrane fractions were prepared for electron microscopy by fixing the pellets in ice cold 2% osmium tetroxide for 24 hr. The samples were rinsed in 2 changes of ice-cold distilled water and then dehydrated in an ethanol series. Small pieces (approximately 1 mm²) were embedded in Epon resin, sectioned and placed on bare 400 mm mesh grids. The sections were then exposed to 1% uranyl acetate for 10 min, rinsed, dried, and finally stained with lead citrate for 1 and one-half min.

Results and Discussion

An initial experiment consisted of determining the relative distribution of ATPase activity in various subcellular fractions and the sensitivity of the enzyme(s) to Mg^{2+} and K^+ (table I). These results illustrate that the majority of the total ATPase remains in the supernatant after a centrifugation of 100,000g for 90 min, however, this ATPase is slightly inhibited by the Mg^{2+} and K^+ . On the other hand the membrane containing fractions, *i.e.*, cell wall, mitochondrial and microsomal fractions, exhibit ATPase activity that is activated by Mg^{2+} and further stimulated by K^+ when Mg^{2+} is also present. The K^+ alone has little effect on the ATPase activity (see table II). Of the 3 fractions exhibiting $\text{Mg}^{2+} + \text{K}^+$ stimulated ATPase activity the mitochondrial and microsomal fractions were highest in specific activity (table I) with the largest amount of total K^+ stimulated activity in the microsomal fraction. The lack of K^+ stimulated ATPase activity in the

Table I. ATPase Distribution Among Subcellular Fractions

Reaction system included 3 mM ATP, 20 mM tris, pH 7.5 and when added 2 mM MgCl_2 and 40 mM KCl. Reaction time was 30 min.

	Crude		Cell wall		ATPase activity Mito.		Micro.		Super	
	Sp. ac. ¹	Total ²	Sp. ac.	Total	Sp. ac.	Total	Sp. ac.	Total	Sp. ac.	Total
Blank	13.0	1035	3.0	6.8	6.8	18.4	6.0	46.6	15.9	1044
Mg^{2+}	12.7	1010	6.3	14.3	13.2	35.8	15.8	122.6	14.1	926
$\text{Mg}^{2+} + \text{K}^+$	15.0	1194	9.5	21.6	22.6	61.2	26.2	203.3	14.3	940

¹ $\mu\text{moles } P_i$ released per mg protein per hr.

² Total $\mu\text{moles } P_i$ released per fraction.

Table II. *Effect of Various Salts on the ATPase Activity of the Cell Wall Fraction*

The reaction mixtures contained 3 mM ATP, 20 mM tris (pH 7.2) and when added 1.5 mM $MgCl_2$ and 50 mM monovalent cations. The reaction time was 30 min at 39°.

Column additions	ATPase activity			Stimulation induced by Mg^{2+}	
	A	B	C	D	
	—Mg	Net	+Mg	Net	D/B×100
	$\mu\text{moles } P_i/\text{mg prot} \times \text{hr}$				%
None	1.74
$MgCl_2$	5.02
KCl	2.22	0.48	10.00	4.98	1038
NaCl	2.32	0.58	9.93	4.91	847
RbCl	2.22	0.48	9.55	4.53	944
CsCl	2.41	0.67	9.27	4.25	634
NH_4Cl	2.60	0.86	9.93	4.91	571
LiCl	2.60	0.86	8.68	3.65	426

soluble fraction has been examined in a variety of ways. The microsomal supernatant was further sedimented by centrifuging at 200,000g for 12 hr and the ATPase activity of the pellet was found not to be stimulated by either Mg^{2+} or $Mg^{2+} + K^+$. Dialysis as well as separations on sephadex were equally ineffective in demonstrating any ATPase sensitivity to Mg^{2+} or K^+ .

The degree of K^+ stimulation in the membrane fractions (with the blank removed the $Mg^{2+} + K^+/Mg^{2+}$ ratio varies from 1.5–3) is quite low relative to some of the $Na^+ + K^+$ stimulated ATPases observed in animal membrane systems, however, it is similar in magnitude to that observed with the majority of animal systems (33). It will be shown later, however, that the total K^+ activated ATPase activity is sufficient to account for K^+ influx in the oat roots.

The effect of various monovalent ions on the ATPase activity of the cell wall fraction is shown in table II. Although KCl resulted in the greatest ATPase activity the other salts were also quite

effective. There was some degree of specificity, however, as evident by the percent monovalent ion stimulation elicited by Mg^{2+} . Although the evidence is meager that Na^+ is actively extruded from higher plant tissues it seemed appropriate in light of the work with animal systems (32,33) and algae (25, 26) to determine whether Na^+ and K^+ effected the ATPase in a synergistic fashion. Table III shows that for both the cell wall and mitochondrial fractions Na^+ is about as effective as K^+ in stimulating the ATPase, however, no significant beneficial effect was observed by their simultaneous presence. Although the data are not shown here it should also be mentioned that ouabain has no effect on the ATPase activity nor does it affect the absorption of K^+ by the oat roots.

The K^+ stimulated ATPase activity could be demonstrated in the presence of either Mg^{2+} or Mn^{2+} but not in the presence of Ca^{2+} (table IV). It was also found that Ca^{2+} inhibited the $Mg^{2+} + K^+$ ATPase activity. Similar results are found with the animal 'transport' ATPase (11). Imidazole was used instead of tris in these experiments, however, no apparent difference existed between these buffers with respect to the K^+ stimulation in the presence of Mg^{2+} .

A variety of commonly employed tissue maceration procedures using various shearing devices did not result in significant alteration in the ATPase

Table IV. *Effect of Ca^{2+} and Ca^{2+} Plus K^+ on the ATPase Activity of the Cell Wall and Mitochondrial Fractions*

The reaction mixture contained 1.5 mM ATP and 20.0 mM imidazole (pH 7.8). The reaction time was 60 min at 39°.

	ATPase activity	
	Cell wall	Mitochondria
	$\mu\text{moles } P_i/\text{mg prot} \times \text{hr}$	
Blank	1.20	1.18
$CaCl_2$ (5 mM)	2.16	5.38
$CaCl_2 + KCl$ (50 mM)	2.11	4.35

Table III. *Effect of Various Combinations of K^+ and Na^+ Concentrations on the ATPase Activity of the Cell Wall and Mitochondrial Fractions*

The concentrations of various additives (except where indicated) were: 3 mM ATP, 1.5 mM $MgCl_2$, 50 mM KCl, 50 mM NaCl and 20 mM tris (pH 7.2). Reaction time was 30 min at 39°.

Additions	ATPase activity	
	Cell wall	Mitochondria
	$\mu\text{moles } P_i/\text{mg prot} \times \text{hr}$	
Blank	1.79	2.88
KCl	2.69	3.39
NaCl	2.35	3.39
$MgCl_2$	4.90	10.20
$MgCl_2 + KCl$	8.55	26.40
$MgCl_2 + NaCl$	8.14	24.60
$MgCl_2 + KCl + NaCl$	7.87	27.90
$MgCl_2 + KCl$ (25 mM) + NaCl (25 mM)	8.41	26.20
$MgCl_2 + KCl$ (75 mM) + NaCl (25 mM)	8.49	27.20
$MgCl_2 + KCl$ (25 mM) + NaCl (75 mM)	7.87	27.30

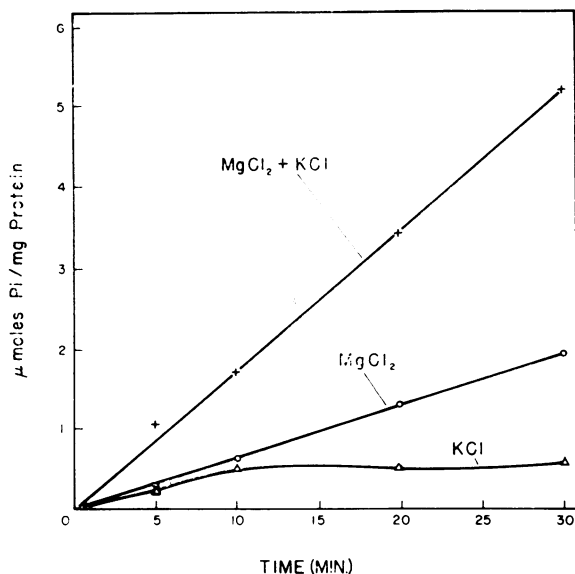


FIG. 1. ATPase activity of the cell wall fraction as a function of time. The reaction mixture contained 3 mM ATP, 20 mM tris (pH 7.2) and when added 1.5 mM $MgCl_2$ and 50 mM KCl.

activity. The sucrose and EDTA concentrations, as well as the pH, of the homogenizing solution were chosen on the basis of preliminary experiments which provided maximum K^+ stimulated ATPase activity. Other additives such as cysteine and deoxycholate were found to be inhibitory and were therefore not included in the grinding medium.

Fig. 1 illustrates that the Mg^{2+} and $Mg^{2+} + K^+$ ATPase activities of the cell wall fraction were

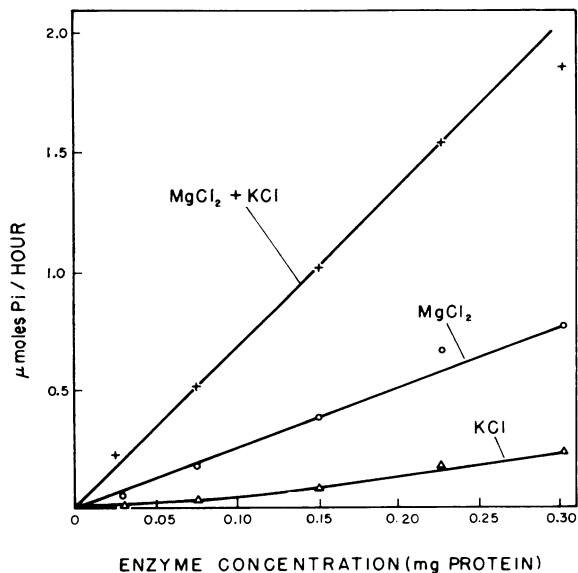


FIG. 2. Effect of enzyme concentration on the ATPase activity of the cell wall fraction. Reaction mixture was the same as for Fig. 1 and the reaction time was 30 min.

linear over a 30 min reaction period. Similar results were obtained with the mitochondrial and microsomal fractions. Fig. 2 shows the effect of enzyme concentration (cell wall fraction) on the rate of the reaction. Again similar results were found with the mitochondrial and microsomal fractions. The pH optimum for the cell wall ATPase was quite high, approximately 8.0 to 8.5 (Fig. 3). Similar results were also found with the mitochondrial and microsomal fractions. The high pH optimum is in contrast to the results obtained by Brown *et al.* (4), Dodds and Ellis (5) and Atkinson and Polya (1) but similar to the report by Gruener and Neumann (15). The temperature optimum was also quite high as shown in Fig. 4, however, this appears to be a characteristic of ATPase enzymes (14). The inset in Fig. 4 shows the K^+ stimulation increases with increasing temperatures over the entire range studied.

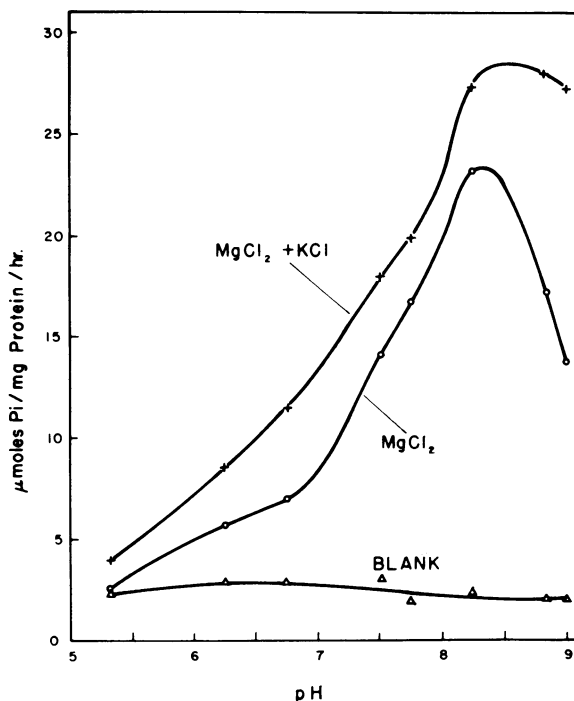


FIG. 3. Effect of pH on the ATPase activity of the cell wall fraction. The reaction mixture contained 3 mM ATP, 100 mM tris-tricine (proportion varied with desired pH) and when added 1.5 mM $MgCl_2$ and 50 mM KCl. Reaction time was 30 min.

Fig. 5 shows the effect of ATP concentration on the rate of the reaction. The optimum concentration is about 4 to 5 mM with higher concentrations being somewhat inhibitory. This, too, is a general characteristic of ATPases and may be due to inhibition of the enzyme by ADP (14) or possibly by the ATP complexing the Mg^{2+} (31). Several phosphorylated substrates were examined to determine whether other phosphatases were activated by monovalent ions.

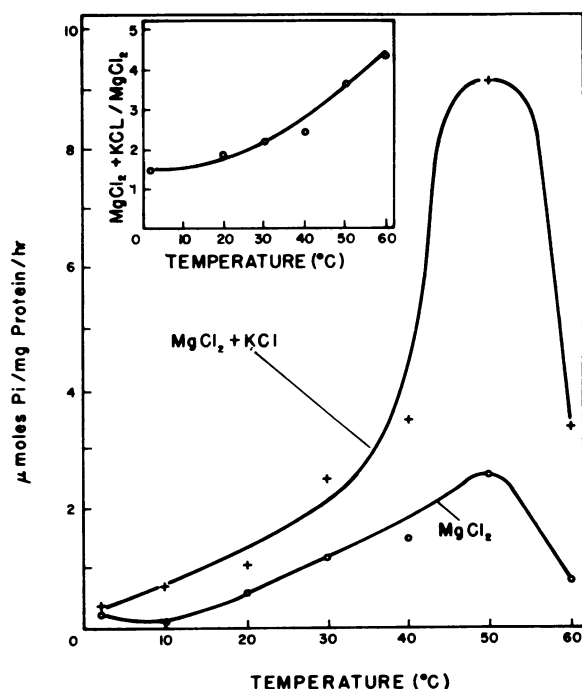


FIG. 4. Effect of temperature on the ATPase activity of the cell wall fraction. Reaction mixture was the same as for Fig. 1 and the reaction time was 30 min. The ordinate in the inset shows the ratio of the ATPase activity in the presence of $Mg^{2+} + K^+$ and Mg^{2+} .

Table V shows that Na^+ (all substrates were used as the Na-salt) causes a significant stimulation of phosphate hydrolysis with both ATP and GTP whereas the release of phosphate from UTP was slightly inhibited.

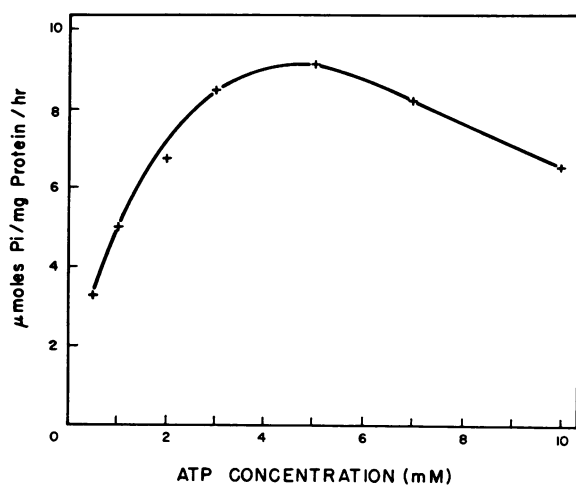


FIG. 5. Effect of ATP concentration on the ATPase activity of the cell wall fraction. The reaction mixture contained 2.5 mM $MgCl_2$, 50 mM KCl, and 50 mM tris (pH 7.1). Reaction time was 30 min.

Table V. Effect of Na^+ on P_i Release From a Variety of Phosphorylated Substrates Using the Cell Wall Fraction

The reaction mixture contained 3 mM substrate (Na-salt), and 20 mM tris (pH 7.1) and when added 1.5 mM $MgCl_2$ and 50 mM NaCl. Reaction time was 30 min at 39°.

Substrate	ATPase activity	
	$MgCl_2$	$MgCl_2 + NaCl$
	$\mu\text{moles } P_i/\text{mg prot} \times \text{hr}$	
Glucose-1-P	0	0
p-Nitrophenyl-P	2.32	3.49
Pyrophosphate	9.18	11.70
AMP	2.32	3.49
ADP	1.63	2.21
ATP	2.79	7.21
CTP	1.16	1.51
GTP	0.81	3.25
UTP	2.44	2.09

The optimum Mg^{2+} (Fig. 6) concentration for the cell wall ATPase was about 4 mM; however, with increasing concentrations of K^+ the optimum Mg^{2+} concentration decreased slightly. The effect of K^+ concentrations on the K^+ stimulated component of the ATPase activity is shown in Fig. 7. For all fractions examined the ATPase activity increases rapidly up to about 10 mM K^+ and then increases more gradually up through 40 mM K^+ .

We have previously reported that low concentrations of oligomycin inhibit K^+ absorption in oat roots (18). It was, therefore, of interest to determine whether the ATPase was sensitive to oligomycin.

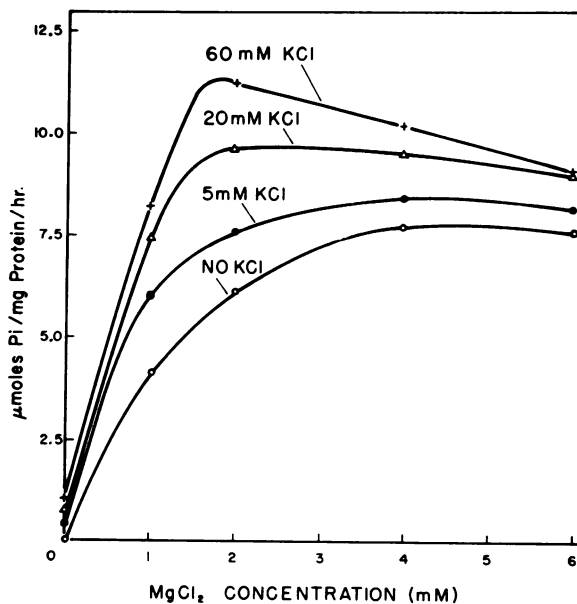


FIG. 6. Effect of $MgCl_2$ and KCl on the ATPase activity of the cell wall fraction. The reaction mixture was the same as for Fig. 1 and the reaction time was 15 min.

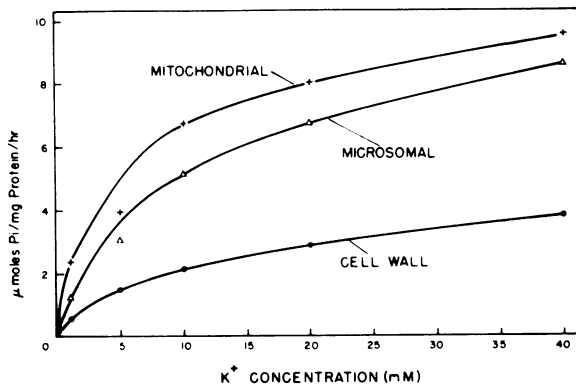


FIG. 7. Effect of KCl concentration on the K⁺ stimulated ATPase activity in the cell wall, mitochondrial and microsomal fractions. The reaction mixture contained 3 mM ATP, 20 mM tris (pH 7.5) and 2 mM MgCl₂. The Mg²⁺ stimulated ATPase activity has been subtracted from the data.

Fig. 8 shows the effect of oligomycin concentrations on both the Mg²⁺ and Mg²⁺ + K⁺ ATPase of the cell wall fraction. At 1 μg/ml oligomycin the Mg²⁺ activated ATPase was inhibited by about 50 %, whereas the Mg²⁺ + K⁺ activated ATPase was inhibited by only about 25 %. The absolute amount of inhibition was basically the same for the Mg²⁺ and Mg²⁺ + K⁺ ATPase which suggests the existence of 2 separate enzymes, one requiring Mg²⁺ and sensitive to oligomycin and the other requiring both Mg²⁺ and K⁺ and being insensitive to oligomycin. In an attempt to evaluate this possibility the sensitivity of the Mg²⁺ and Mg²⁺ + K⁺ ATPase to oligomycin in all fractions was examined. The results are shown in table VI and they illustrate that the various fractions were differentially sensitive to oligomycin. The general distribution of the oligomycin sensitivity of the Mg²⁺ + K⁺ ATPase activity is further illustrated in Fig. 9. The mitochondrial fraction is most sensitive to oligomycin and the microsomal fraction is least sensitive to oligomycin. These results are indeed suggestive that at least 2 ATPases are being measured.

Because of the apparent presence of more than 1 ATPase in each fraction it seemed desirable to assess the heterogeneity of the various fractions with the electron microscope (plates I, II, and III). Although not apparent in plate I, the cell wall fraction was found to contain, in addition to wall fragments, some intact mitochondria as well as empty vesicles, however, in the case of the microsomal fraction (plate III) no intact mitochondria appear to be present. The mitochondrial fraction (plate II), in addition to containing mitochondria, also contains some vesicular and spiral-like structures which could perhaps represent either mitochondrial membranes (28), endoplasmic reticulum or fragments of plasma membrane of fairly large size. Plate IV

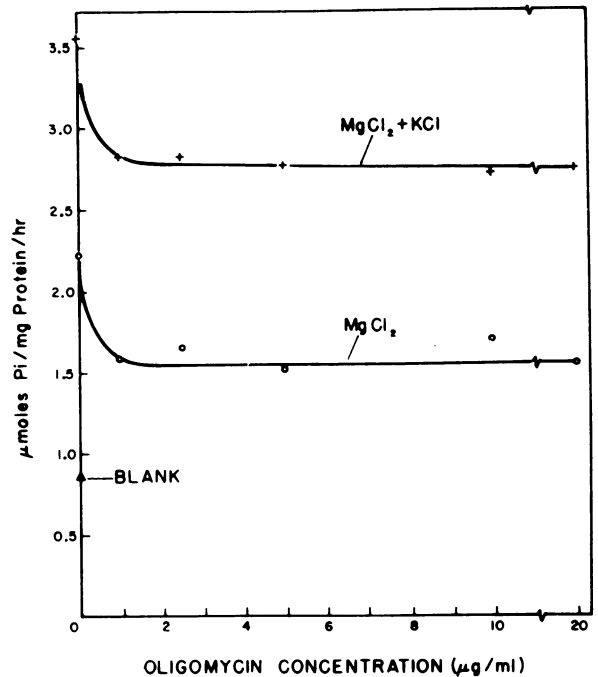
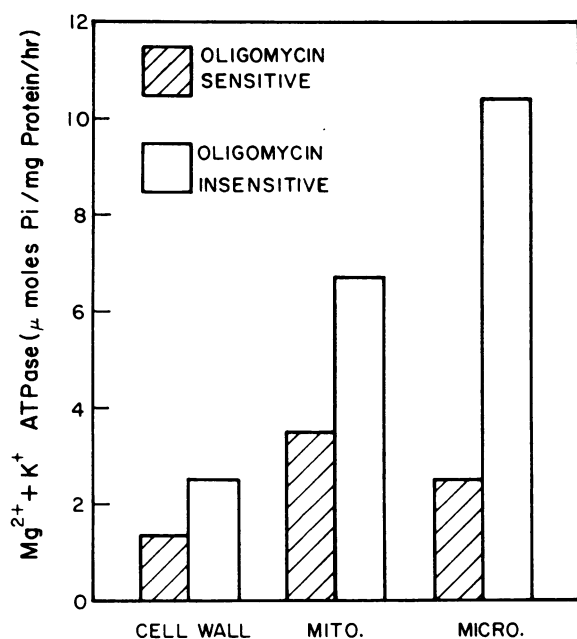


FIG. 8. Effect of oligomycin on the MgCl₂ and MgCl₂ + KCl stimulated ATPase activity of the cell wall fraction. The reaction system was the same as for Fig. 1 and the reaction time was 30 min.

Table VI. Effect of Oligomycin on the Mg²⁺ and Mg²⁺ + K⁺ Stimulated ATPase in the Cell Wall, Mitochondrial, and Microsomal Fractions

Reaction mixture consisted of 3 mM ATP, and 20 mM tris (pH 7.5) and when added 1.5 mM MgCl₂, 50 mM KCl, and 1.25 μg/ml oligomycin. Oligomycin was added in absolute ethanol and all treatments contained equivalent amounts of ethanol. Reaction time was 30 min. The blank ATPase activity (*i.e.* the activity in the absence of added salts) has been subtracted.

Line		ATPase activity		
		Cell wall	Mitochondria	Microsome
		μmoles P _i /mg prot × hr		
1	Mg ²⁺	1.38	3.97	5.86
2	Mg ²⁺ + Oligomycin	0.83	2.32	4.29
3	Mg ²⁺ + K	4.00	9.87	12.97
4	Mg ²⁺ + K ⁺ + Oligomycin	2.51	6.72	10.43



SUBCELLULAR FRACTIONS

FIG. 9. The distribution of oligomycin sensitive and insensitive $Mg^{2+} + K^{+}$ ATPase in the various membrane fractions. See table VI for reaction system.

shows a higher magnification of 1 of the spiral-like structures contaminating the mitochondrial fraction. The absence of the spiral-like structures in the microsome fraction probably means that the membrane fragments are sufficiently small in size that they form only the single membrane vesicles. At least it would appear that all fractions do contain a variety of membrane structures and the existence of more than 1 ATPase associated with different membrane structures in each fraction would be quite possible.

At this point in our investigations it is unknown whether the monovalent ion stimulated ATPase(s) associated with the various membrane fractions has any relationship to ion transport. This being the case, it seemed desirable to know whether sufficient monovalent ion stimulated ATPase existed to account for the absorption of ions by the root. With this objective in mind we determined, at a variety of K^{+} concentrations, the energy dependent absorption of K^{+} per gram fresh weight and with a separate but identical batch of roots the total K^{+} stimulated ATPase in all subcellular fractions per gram fresh weight of tissue. In order to determine the total K^{+} stimulated ATPase activity, the Mg^{2+} stimulated activity was subtracted from the $Mg^{2+} + K^{+}$ stimulated activity in each fraction, and then the K^{+} stimulated ATPase activities from each fraction were added together. A complicating factor involved in this type of analysis is determining the extent of homogenization. In other words, not all membranes

are released during the grinding in a mortar and pestle. In order to estimate the extent of membrane release during grinding the phospholipid contents of unground roots and the crude homogenates were determined. These results, assuming all the phospholipid to be present in membranes, indicated that 42% of the membranes were released during the grinding. (The average values from the 3 experiments used in this analysis were $1.46 \mu\text{moles lipid P/g fresh wt}$ in unground roots and $0.62 \mu\text{mole lipid P/g fresh wt}$ in the crude homogenates.) On the basis of the phospholipid data it was then possible to estimate more closely the total K^{+} stimulated ATPase per g fresh weight of roots. These data for several concentrations of K^{+} are shown in Fig. 10. The K^{+} absorbed per K^{+} stimulated ATPase at all K^{+} concentrations investigated were in the range of 0.7 to 0.8. If one includes only the oligomycin insensitive, K^{+} stimulated ATPase for the calculations, the range in values is from 0.89 to 1.14 with an average value for all K^{+} concentrations of 1.04. If only the oligomycin sensitive ATPase is included for the calculations, the range in values is from 2.40 to 3.08 with an average value for all K^{+} concentrations of 2.81. Now whether this indicates that sufficient ATPase exists to account for transport is debatable since no data exist for plant tissues concerning ions transported per ATP hydrolyzed. However, for most animal tissues it has been found that 2 to 3 moles of K^{+} or Na^{+} are transported per mole of ATP hydrolyzed (32, 34). Using this as a standard it would seem safe to conclude that there is sufficient K^{+} stimulated ATPase to account for the K^{+} absorbed in oat roots.

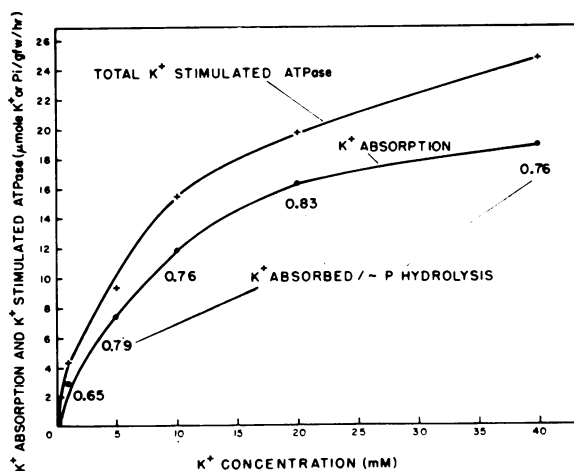


FIG. 10. Effect of KCl concentrations on the absorption of K^{+} by oat roots and on the total K^{+} stimulated ATPase of oat roots. The reaction mixture for the ATPase assay contained 3 mM ATP, 20 mM tris (pH 7.5) and 2 mM $MgCl_2$. The reaction time was 30 min.

General Discussion

The present study represents an attempt to determine the existence and characteristics of ATPase(s) from plant roots which may participate in ion transport reactions. The basic findings are that the various membrane fractions examined (cell wall, mitochondrial and microsomal) do possess ATPase activity. The ATPases from all membrane fractions require Mg^{2+} or Mn^{2+} but are further stimulated by a variety of monovalent salts. The monovalent salts are ineffective in the absence of the activating divalent cation. The specific activities of the ATPases in the various fractions were approximately the same, generally being slightly higher in the mitochondrial and microsomal fractions. The microsomal fraction, however, contained more total ATPase activity than the other fractions. The supernatant remaining after the microsomal centrifugation was very high in ATPase activity, however, it was not stimulated by either Mg^{2+} or the monovalent salts. In fact, the soluble ATPase activity was generally inhibited by the salts.

The concentrations of monovalent salts required to maximally activate the ATPase(s) were quite high (Fig. 7 and 10). With respect to a possible relationship to ion transport, these high concentrations would be in the range of mechanism 2 as described by Epstein (10) and Torri and Laties (35). Whether a significant activation occurs at the lower concentrations (their mechanism 1 system), which becomes saturated at approximately 0.2 mM, has yet to be determined. Also with respect to a possible relationship to transport it is encouraging that the ATPase does exhibit a certain degree of specificity regarding the monovalent ions (table II). In general, the effectiveness of the various ions is similar to their rates of absorption in roots of other species (13). However, before a definite relationship or correlation between the ATPase and transport can be established it will be necessary to determine the actual kinetics of the ion stimulations of the enzyme as well as the kinetics of ion transport for several different monovalent ions and with 1 particular plant species.

Oligomycin was found to inhibit the Mg^{2+} and $Mg^{2+} + K^{+}$ ATPase activity to different extents and the percentage inhibition was different in the various membrane fractions (table VI and Fig. 8 and 9). From these results it would appear that we are dealing with more than 1. membrane associated, ATPase. On the basis of other studies (17) the mitochondria would be expected to contain an oligomycin inhibited ATPase and this fraction was indeed most sensitive to oligomycin (Fig. 9). However, the microsomal fraction also contained some oligomycin sensitive ATPase and on the basis of the electron micrographs (plate III) there did not appear to be any mitochondria contaminating this fraction. Thus it is quite likely that a non-mitochondrial,

oligomycin sensitive ATPase also exists. Obviously it is impossible with the present data to designate either the oligomycin sensitive or insensitive ATPase activity as being more likely to be related to ion transport. Because of the nature of the oligomycin inhibition of ion absorption in roots (18,21) it was anticipated that a transport related ATPase would be inhibited by this antibiotic. However, from the results in table VI it can be deduced that the oligomycin insensitive ATPase is stimulated more by K^{+} than is the oligomycin inhibited enzyme. It would also seem to be pertinent that this activity was highest in the microsomal fraction inasmuch as the animal 'transport' ATPase also generally sediments in this fraction (33). Wallach (36) has shown conclusively, however, that the microsomal vesicles containing $Na^{+} + K^{+}$ ATPase of animal cells were derived from the plasma membranes.

All the fractions, but particularly the mitochondrial and microsomal fractions (plates II, III, and IV), were very heterogeneous with regard to types of membrane constituents. The apparently empty vesicular structures in both these fractions as well as the spiral-like structures in the mitochondrial fraction are of particular interest since they closely resemble isolated bacterial plasma membranes (30). We are currently attempting to purify these vesicles using density gradient centrifugation and to examine them for ATPase activity and sensitivity to various ions and oligomycin.

One of the major differences between the work reported here and other reports of ion stimulated ATPases of plant origin (1,4, and 5) is the difference in pH optima. At present the reasons for these differences are unknown but it is interesting that we have found oligomycin to be a more effective inhibitor at pH 7.5 than at pH 6.5. Although not the only possible interpretation, this raises the possibility that the oligomycin sensitive and insensitive ATPase may have different pH optima. Should this be the case, the reported differences could perhaps be reconciled.

Although it is unclear whether the monovalent ion stimulated ATPase activities reported here are in fact related to ion transport, it does appear that sufficient K^{+} stimulated ATPase exists in the cell to account for the observed rates of K^{+} transport (Fig. 10). Further experiments of the type shown in Fig. 10, using different plant species which vary in transport capacity over a wide range, should help to establish whether the enzyme is indeed related to ion transport.

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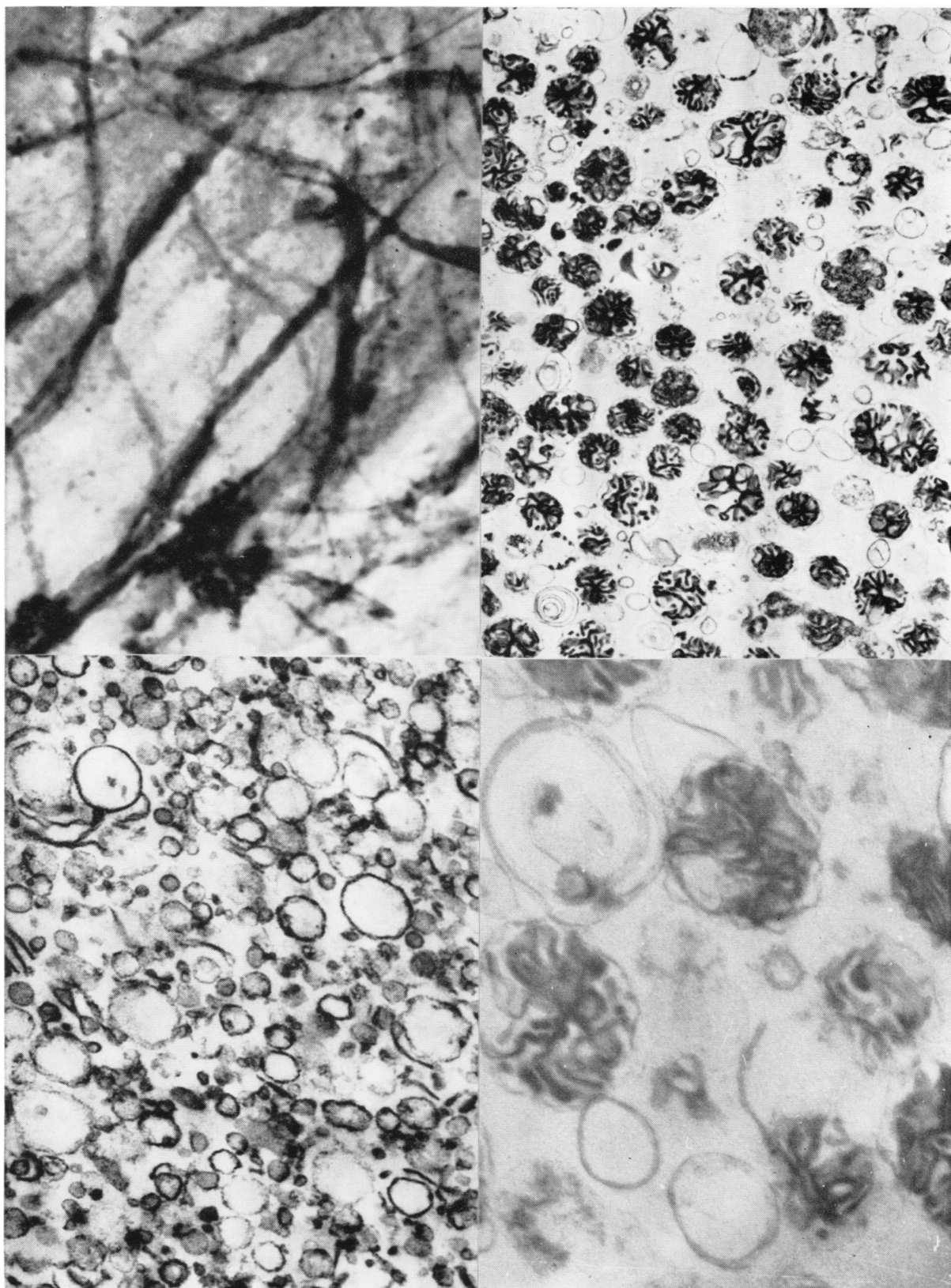


FIG. 11. (Upper left). Cell wall fraction. Magnification - $\times 25,000$. See Materials and Methods for techniques involved in all 4 electron micrograph plates. (Upper right). Mitochondrial fraction. Magnification - $\times 10,250$. (Lower left). Microsomal fraction. Magnification - $\times 59,400$. (Lower right). Higher magnification of the mitochondrial fraction showing spiral-like structures. Magnification - $\times 47,400$. This section was unstained.

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